APHIDS AND OTHER HEMIPTEROUS INSECTS | VOL.17 (2011) 121–127

Effect of spent hop extracts on the probing and settling behaviour of *Myzus persicae* (Sulzer, 1776)

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ABSTRACT

The aim of the present study was to evaluate the effect of four different spent hop (*Humulus lupulus* L.) extracts on a peach potato aphid *Myzus persicae* (Sulzer, 1776) behaviour during settling on plants and stylet penetration. None of the extracts produced an immediate and direct effect on aphid probing and feeding. However, extract 4 had a delayed deterrent effect on aphid settling although it did not impede aphid probing and sap ingestion. It is likely that extract 4 contained a component that could reach sieve elements and produce a postingestional metabolic deterrent effect without evoking the rejection behaviour based on gustatory stimuli.

KEY WORDS: probing behaviour, stylet penetration, EPG, antifeedants

INTRODUCTION

A common hop (*Humulus lupulus* L.) (Cannabinaceae) is a natural component of riverside wetland forests of the temperate northern hemisphere (Prieditis, 1997; Matuszkiewicz, 2002). The female strobilous inflorescences ('hops' or 'cones') of a hop contain more than 1000 chemicals displaying a high biological activity including antimicrobial, oestrogenic, and anticancerogenic characteristics (Chadwick *et al*., 2006; Langezaal *et al*., 1992; Farago *et al*., 2009; Wang *et al*., 2008).

Gökçe *et al*. (2009) found that hop extracts were toxic to and deterred the feeding of larval obliquebanded leafroller *Choristoneura rosaceana* (Harris, 1841). Powell *et al*. (1997) demonstrated that hop acids inhibited the settling of two cereal aphid species, a grain aphid *Sitobion avenae* (Fabricius, 1775) and a bird cherry oat aphid *Rhopalosiphum padi* (Linnaeus, 1758).

Hops have been cultivated for more than 1000 years and since ancient times, hops and hop extracts have been used as bittering agents in the beer brewing process (Chadwick *et al*., 2006). An important by-product of hop extraction are spent hops. Spent hops contain traces of bitter acids and essential oils, e.g., β-myrcene, limonene, linalool, geraniol, 2-undecanone, β-caryophyllene, α-humulene, and caryophyllene (Anioł *et al*., 2007). Some of these terpenes had a repellent and deterrent effect on a peach potato aphid *Myzus persicae* (Sulzer, 1776) (Gabryś *et al*., 2005).

The aim of the present study was to evaluate the effect of four different spent hop extracts on the peach potato aphid behaviour during settling on plants and during stylet penetration.

MATERIAL AND METHODS

After supercritical CO_2 extraction, spent hops (*H. lupulus* var. Marynka, 2005) were prepared and extracted as described previously (Anioł & Żołnierczyk, 2008). Thus, the spent hops (10 g) were refluxed with 100 ml of methanol in a 500-ml round-bottom flask. After 30 min. the mixture was cooled down with cold water and rapidly filtered. The solvent was evaporated under vacuum and the residue was dissolved in ethanol to obtain extract 3. Soxlet extractions were performed in hexane for 180 min (extract 4), 360 min. (extract 2) and in methanol for 360 min. (extract 1). Methanol was chosen as a very good extractant of polar compounds and hexane was selected for the extraction of unpolar constituents from spent hops. As a result, two extracts containing lipophilic components (extracts 2 and 4) and two extracts with hydrophilic components (extracts 1 and 3) were obtained. The extracts were applied on the surface of the test leaves using a fine paint brush for uniform distribution. All biological tests were performed 1 hour after the application of the compounds to allow the evaporation of the solvent.

Aphids (*M. persicae*) and plants (Chinese cabbage *Brassica pekinensis* (Lour.)) were reared in the laboratory at 20°C, 65% r.h., and L16:8D photoperiod. Young, 2-3 days old viviparous apterous females were selected for experiments. Cabbage plants used in the bioassays were 5-6 weeks old.

Aphid settling was assessed using the half-leaf choice-test: compounds were applied on one half of the leaf, while the other side of the midrib was coated with ethanol and acted as a control. Aphids that settled on each side of the midrib were counted at 15', 30', 1h, 2h, and 24h intervals after being given access to the leaf

(8 replicates, 20 adult apterous aphids/replicate). From the data thus obtained the relative index of preference (IP) was calculated using the formula according to Nawrot *et al.* (1982): IP = (C-T/C+T) , where C denotes the number of aphids settled on the control half of the leaf and T – the number of aphids settled on the extract-treated half of the leaf. The results were statistically analysed using analysis of variance. If aphids showed significant preference to the half of the leaf treated with the studied extract (IP<0), the extract was described as having attractant properties, whereas if aphids settled mainly on the control half of the leaf (IP>0), the extract was identified as a deterrent.

Initial aphid responses were studied by direct observation of the freely moving aphids on a leaf treated with the studied extracts, using a video camera. The experiment was carried out for 15 minutes continuously (16 aphids/extract). The time spent on the leaf and the duration of probing were recorded on basis of the relationship between antennal and body movements and penetration of the stylets as described by Hardie *et al.* (1992). The position of antennae parallel to the abdomen and the cessation of body movements were associated with stylet penetration. The results were statistically analysed using analysis of variance.

Aphid probing was monitored using the technique of electronic registration of aphid probing in plant tissues, known as EPG (Tjallingii, 1995a). In this experimental set-up, an aphid and a plant are made parts of an electric circuit, which is completed when the aphid inserts its stylets into the plant. Weak voltage is supplied within the circuit, and all changing electric properties are recorded as EPG waveforms that can be correlated with aphid activities and stylet position in plant tissues. Aphids were attached to a golden wire electrode with conductive silver paint and starved for 1h prior to the experiment. Probing behaviour of 16 apterous females per studied plant/aphid combination was monitored for 8h continuously with a four-channel DC EPG recording equipment. Each aphid was given access to a freshly prepared plant: one leaf of the plant was covered with the studied extract or a solvent (control). The plant electrode was placed in the soil. Signals were saved and analysed using the PROBE 3.1 software provided by W. F. Tjallingii (www. epgsystems.eu). The parameters derived from EPGs were analysed according to their frequency and duration in configuration related to activities in peripheral and vascular tissues. The values of EPG parameters were analysed using Mann-Whitney U test.

RESULTS AND DISCUSSION

The number of aphids that settled on the leaf section treated with any of the spent hop extracts changed in the course of time. Among the aphids, there was a tendency to select the leaves treated with extracts 1, 2, and 3 (IP<0), as opposed to leaves treated with the extract 4 (IP>0). However, the significant preference for

the leaves treated with the extract 1, 2, and 3 occurred only during the first two hours of the experiment, whereas the significant non-preference for the leaves treated with extract 4 was observed from the second hour of the experiment until the end of experiment (data not shown). These results indicate that none of the extracts produced immediate, direct effect on aphid probing and feeding. The positive effect of extracts 1, 2, and 3 was short-lived and the negative effect of extract 4 was delayed but relatively strong and durable.

The direct observation of aphid behaviour showed that there were no significant differences in the initial responses of aphids on control and extract-treated spent hop leaves. During the initial 15 minutes since being given access to the leaves, no aphid avoided the extract-treated ones. The total time spent on the treated leaves ranged from 88 to 95% of the time spent on control leaves. The total probing time, frequency and mean duration of probes (3-5 minutes) were similar in all aphids irrespective of a treatment applied (data not shown), which meant that these probes reached into deeper layers of mesophyll (Gabryś *et al*., 1997).

The stylet penetration, i.e., the probing and feeding behaviour of *M. persicae* was studied after the application of spent hop extract 4 that was the only one to show deterrent properties in aphid settling bioassay.

The total time that aphids spent on probing in peripheral tissues (pathway activity) accounted for 60 and 84 percent of the experimental time on control and plants treated with extract 4, respectively. On control plants, 70% of that time was pathway activity, and 21% – phloem phase activity, whrereas on the treated ones – 74 and 18%, respectively. The xylem sap ingestion engaged the remaining probing time. The total number of probes on control leaves was 17 times greater than on extract-treated ones. However, the proportion of probes that ended in the phloem vessels was much lower on control than on treated leaves (16 and 70%, respectively). The total time preceding the first phloem phase was similar in all aphids (2.3 – 3.5 hours on average). However, the non-probing activities occupied significantly less of that time on control than on extract-treated leaves. Before the first phloem phase, aphid probing was rarely interrupted and the probes were relatively long on extract-treated leaves (1.6 hour long, on average), which was in contrast to the probes on control plants where short, 2-10 min., and numerous probes predominated. During the phloem phase, no significant differences were found between aphids on the control and extract-treated plants, except the total duration of salivation during the phloem phase which was significantly longer on extract-treated plants than on control plants (Tab. 1).

The duration of the phloem sap ingestion period and the amount of the consumed sap are positively correlated. It has been demonstrated that aphids ingest sap in a continuous, passive, and steady manner and control the amount of imbibed sap by regulating the duration of the ingestion period (Tjallingii, 1995b). The continuation or termination of probing and/or sap ingestion depends mainly on allelochemicals that may be present in plant cells (Czerniewicz *et al.*, 2008). Considering the

long and uninterrupted probing and E2 periods, it may be assumed that aphids on extract-treated plants were motivated to probe and, in consequence, consumed a considerable amount of sap. This, in turn, implies the absence of feeding deterrents both in mesophyll and phloem. However, in the settling choice assay, aphids clearly preferred control leaves to extract 4-treated leaves for at least 24 hours after application. It is likely that extract 4 contained a component that could reach sieve elements using the symplastic or apoplastic pathway and produce a delayed, postingestional metabolic detrrent effect without evoking the rejection behaviour based on gustatory stimuli (Frazier & Chyb, 1995). Lipophilic compounds can disrupt various biochemical and physiological processes by affecting structure and functions of cell membranes (Sikkema *et al*., 1995). Exogenously applied hop chemicals, alpha- and beta-hop acids and colupulone, had an antifeedant effect on *S. avenae* and *R. padi* (Powell *et al.,* 1997). Nevertheless, it remains to be studied what is the composition of spent hop extract 4 and what is the role of individual compounds in evoking *M. persicae* response to its presence.

Table 1. Probing activities of a peach potato aphid *Myzus persicae* (Sulzer, 1776) after the application of spent hop extract 4

EPG parameters		Control	Extract 4	p
General aspects of aphid behaviour				
Total duration of probing	h	4.8 (±0.4)	6.7 (±0.6)	0.0024
Total duration of pathway	h	3.3 (±0.3)	5.0 (±0.5)	0.0102
Total duration of xylem phase	h	0.4 (±0.2)	0.3 (±0.1)	0.7319
Total duration of phloem phase	h	1.1 (±0.3)	1.4 (±0.4)	0.7319
Total duration of phloem sap ingestion phase	h	0.9 (±0.2)	1.0 (±0.3)	0.5680
Proportion of phloem phase in total probing	%	21.2 (±4.0)	17.6 (±5.0)	0.1454
Number of probes	#	26.9 (±4.2)	1.6 (±0.4)	0.0000
Number of probes with phloem phase	#	4.2 (±0.9)	1.1 (±0.3)	0.0013
Activities in peripheral tissues before phloem phase[1]				
Time from 1st probe to 1st phloem phase	h	2.3 (±0.6)	3.5 (±0.5)	0.1266
Duration of non-probing before 1st phloem phase	h	0.8 (±0.2)	1.0 (±1.0)	0.0067
Number of probes before 1st phloem phase	#	9.6 (±2.3)	1.5 (±0.4)	0.0001
Probes <2 min.	#	4.3 (±1.1)	0.2 (±0.2)	0.0001
Probes 2-10 min.	#	3.6 (±1.0)	0.3 (±0.2)	0.0012
Probes >10 min.	#	1.7 (±0.5)	0.9 (±0.1)	0.5206
Activities in sieve elements[1]				
Duration of 1st phloem phase	h	0.8 (±0.2)	0.2 (±0.1)	0.7972
Duration of 1st phloem sap ingestion phase	h	0.1 (±0.1)	0.2 (±0.1)	0.8528
Duration of 1st salivation phase	min	0.8 (±0.2)	3.0 (±1.9)	0.4123
Time from 1st phloem phase to 1st sustained sap ingestion phase	min	2.5 (±0.6)	2.3 (±0.7)	0.5107
Total duration of salivation during phloem phase	h	0.2 ±0.06)	0.4 (±0.3)	0.0279

Values represent means ±SE. [1]Only aphids that showed a phloem phase (E1), phloem sap ingestion phase (E2), or sustained sap ingestion phase (E2>10 min.) were included in statistical analysis and if an aphid did not show one of these phases, the value of a given parameter for such individual was zero; statistically significant difference in comparison to control at $p<0.05$; Mann-Whitney U-test

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Wpływ ekstraktów z wychmielin na zachowanie mszycy brzoskwiniowej - *Myzus persicae* (Sulzer, 1776) podczas zasiedlania roślin i żerowania

STRESZCZENIE

Celem pracy była ocena wpływu czterech różnych ekstraktów z wychmielin na zachowanie mszycy brzoskwiniowej *Myzus persicae* (Sulzer, 1776) podczas zasiedlania roślin i żerowania. Żaden z badanych ekstraktów nie miał natychmiastowego i bezpośredniego wpływu na zachowanie mszyc podczas penetracji kłujki i żerowania. Niemniej jednak, ekstrakt 4 spowodował opóźnioną negatywną reakcję mszyc podczas zasiedlania roślin. Prawdopodobnie, ekstrakt 4 zawierał składnik migrujący do elementów sitowych, o deterentnym działaniu uwidaczniającym się dopiero po spożyciu soku floemowego przez mszyce.